agronomie (1993) 13, 865-876
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Plant breeding

Brown-midrib genes of maize: a review

Y Barrière*, O Argillier

INRA, Station d'Amélioration des Plantes Fourragères, F86600 Lusignan, France

(Received 21 May 1993; accepted 23 September 1993)

Summary — The efficiency of forage for ruminant animals primarily depends on cell-wall constituent content, mostly cellulose and hemicellulose, and on their digestibility, mostly reduced by lignin incrustration. Four brown-midrib genes (*bm1*, *bm2*, *bm3* and *bm4*) have been described in maize. Brown-midrib plants were described as having a lower lignin content than normal genotypes; the ratios of hydroxycinnamic acids released after alkaline hydrolysis, and the ratios between monomeric units released after alkaline nitrobenzene oxidation or thioacidolysis also differed in normal and brown-midrib plants. The *bm3* gene, allowing an important decrease of lignin content, and a better improvement in plant digestibility, was also the most studied. When cattle were fed *bm3* plants, intake and digestibility were higher compared to normal isogenic ones, and the rate of digestion was also higher. However, the agronomical value of the brown-midrib genotypes was distinctively lower than that of their normal isogenic counterparts and this was particularly notable on the *bm3* gene. This observation also appeared to be genotype-specific, suggesting the possibility of producing brown-midrib genotypes that are practically as good as the normal ones, but only when using well-adapted breeding methods, with normal lines of a very high agronomical value.

maize / brown-midrib genes / feeding value / lignin / agronomic value

Résumé — Les gènes *brown-midrib* chez le maïs. Revue bibliographique. *L'utilisation des fourrages par les ruminants dépend essentiellement de la teneur en constituants pariétaux, principalement cellulose et hémicelluloses, et de leur digestibilité, réduite particulièrement par la présence de lignines dans ces parois. Quatre gènes* brown-midrib *(*bm1, bm2, bm3, bm4*) ont été mis en évidence chez le maïs. Les plantes* brown midrib *diffèrent des maïs normaux, particulièrement par une teneur réduite en lignines, par des rapports modifiés, entre les acides hydroxycinnamiques libérés après hydrolyse alcaline des lignines, et par des rapports également modifés entre les unités monomériques libérées après oxydation alcaline au nitrobenzène ou thioacidolyse de cette lignine. Le gène* bm3, *qui conduit à une forte réduction de teneur en lignines et surtout, résultat obtenu dès les premières mesures de digestibilité* in vitro, *à la plus importante augmentation de digestibilité, a fait l'objet du plus grand nombre d'études. Cela a permis de confirmer que les génotyes* bm3 *étaient mieux ingérés par les ruminants, avaient,* in vivo, *une meilleure digestibilité, avec une vitesse de digestion plus élevée. En revanche, la valeur agronomique des maïs* brown-midrib *est plus faible que celle des maïs normaux, particulièrement en ce qui concerne le gène* bm3. *Cependant la variabilité génétique mise en évidence pour des caractères agronomiques suggère qu'il doit être possible d'obtenir des génotypes* bm3 *pratiquement du niveau des génotypes normaux, à condition d'employer des méthodes de sélection adaptées, et en n'introgressant que les lignées normales de très bon niveau agronomique.*

maïs /* brown-midrib */ valeur alimentaire / lignine / valeur agronomique

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* Correspondence and reprints

INTRODUCTION

According to Jorgenson (1931), the first brown-midrib maize plants appeared in a 1-yr self-pollinated line of north-western dent corn, at the University Farm, St Paul, Minnesota, in 1924. The brown-midrib maize plants exhibit a reddish-brown pigmentation of the leaf midrib, that becomes visible in plants at the 4–6 leaf stage. The pigmentation appears in the stem with its lignification visually associated with rind and vascular bundles. Whilst maturing, the coloration fades and may disappear on leaves, but remains in the stalks.

The first studies of linkage relations were done by Eyster (1926), but most of the morphologic and genetic descriptions of this type were made by Jorgenson (1931). Subsequently, this gene was named *bm1*, and 3 other genes inducing the brown-midrib phenotype were described, *bm2* (Burnham and Brink, 1932), *bm3* (Emerson *et al*, 1935) and *bm4* (Burnham, 1947). These 4 genes originated from natural mutations. They are not alleles, and each segregates as a simple Mendelian recessive character. They were located on chromosomes 5, 1, 4 and 9 of maize respectively, *bm1* and *bm3* near the respective centromers, and *bm2* and *bm4* close to the respective extremities of the longer arms (Neuffer *et al*, 1968).

Approximately 40 yr after their discovery, the brown-midrib mutations appeared to have a drastic effect on lignin quantity and quality, and later still, to improve the digestibility of plants in ruminants. Natural or artificial brown-midrib mutants of maize, sorghum, sudangrass or pearl millet also offer a unique model for studying lignification, cell-wall phenolics, carbohydrates complexes and utilization of forage by rumen micro-organisms. A synthesis of the potential of brown-midrib mutants for improvement of C4 graminaceous forage quality has been described by Cherney *et al* (1991). The present paper is devoted to an extensive discussion of studies concerning brown-midrib mutants of maize, with chronological and thematic standpoints; a synthesis is attempted in the final part of this text. The first studies performed on brown midrib dealt with lignin content and composition, and feeding value estimated by *in vitro* digestibility in order to compare normal plants and often the various brown-midrib mutant genes *bm1*, *bm2*, *bm3* and *bm4*. Later, the studies were essentially concerned with *bm3* maize plants and to a lesser extent with *bm1*; cell-wall constituent contents and compositions were measured, feeding values were estimated by *in vitro* and *in vivo* digestibility, and agronomic values and mechanical properties were observed.

LIGNIN BIOCHEMICAL COMPOSITION

Some data on lignin biochemical composition, regardless of the plant species, but more specifically for gramineous plants, should be discussed before further investigating maize brown-midrib materials. Lignins are tridimensional molecules resulting from the polymerization of 3 hydroxycinnamic alcohols. These alcohols originate from 3 hydroxycinnamic acids, *p*-coumaric acid, ferulic acid and sinapic acid. The monomeric phenylpropane units are linked through ether or carbon-carbon linkages to build the lignin. The most frequent linkage is the β-O-4 ether linkage (Monties, 1989; Lewis and Yamamoto, 1990; Lapierre, 1992). Alkaline nitrobenzene hydrolysis of the lignin releases 3 aldehydes, *p*-hydroxybenzaldehyde, vanillin and syringaldehyde. Hydroxycinnamic acids are also directly bound to lignin: *p*-coumaric acid is mostly esterified (ester bonds are alkali-labile) and ferulic acid esterified or etherified. Ferulic acid and some *p*-coumaric acid are bound to hemicellulose through an ester linkage to lateral arabinose chains of arabinoxylans (Kato and Nevins, 1985; Mueller-Harvey and Hartley; 1986; Nishitani and Nevins, 1987; Hartley *et al*, 1990; Ishii *et al*, 1990). These 2 acids could therefore cross-link lignin and hemicellulose (Morrison, 1974; Burritt *et al*, 1984; Scalbert *et al*, 1985; Jung, 1989; Iiyama *et al*, 1990; Kondo *et al*, 1990). For instance, Helm and Ralph (1991) reported the possibility of an ether linkage between lignin and ferulic acid, and of an ester linkage between this ferulic acid and an arabinoxylan.

COMPARISON OF LIGNIN CONTENT AND COMPOSITION BETWEEN NORMAL AND BROWN-MIDRIB MAIZE

Lignin content

Kuc and Nelson (1964) first pointed out that the stalks and leaves of *bm1* plants had a lower Ross–Potter lignin content than non-mutant plants (18.5 and 21.8% of the cell-wall residue, respectively, average values of 4 sampling times after sowing). The 4 brown-mibrid genes were studied a few years later by Kuc *et al* (1968) (*bm1*, *bm2*, *bm3*, *bm4* and the double recessive mutants), who noted a difference in content of between 3.0 to 3.5 percent unit of dimethylformamide (DMF) lignin in the stalk cell wall of normal plants and brown-midrib mutants, but no significant difference between the various brown-midrib genes. However, the double mutants had an even smaller lignin content. Lechtenberg *et al,* (1972) re-compared the 4 *bm1*, *bm2*, *bm3* and *bm4* genes with the Troyer red (Tr) inbred line, which was harvested at 35 and/or 55 d after silking. Hybrid pools, segregating for the brown-midrib mutants *bm1*, *bm2*, *bm3*, were simultaneously studied. The smallest acid detergent lignin (ADL) content of stover dry matter was only found in the genotypes *bm3* and *bm1bm3* whereas the other genotypes shared similar values to those of the normal genotypes. Muller *et al* (1971) who also worked with the Tr inbred, established that the ADL content in plants harvested 35 d after flowering, was lower in the *bm3* plants (4.4%) than in the *bm1bm3* (4.6%), the *bm1* (5.1%) and the normal plants (6.1%). ADL of the leaf blades and sheaths in normal plants was also higher than in the 3 other genetic types; ADL in the stalks of the *bm3* and *bm1bm3* was lower than in the *bm1* and normal plants. Whilst studying the Tr inbred and its isogenic mutant *bm1*, Cymbaluk *et al* (1973) found that the *bm1* stalks were poorer in AOAC lignin content (sulphuric acid lignin in dry matter).

All the subsequent studies performed with normal and *bm3* maize plants showed reduced contents of lignin in *bm3*, regardless of the methods of analysis used (Kuc *et al,* 1968; Muller *et al,* 1972; El-Tekriti *et al,* 1976; Frenchick *et al,* 1976; Keith *et al,* 1981; Stallings *et al,* 1982; Weller *et al,* 1984; Grand *et al,* 1985; Gupta *et al,* 1988; Inoue and Kasuga, 1989, 1990; Grenet and Barry, 1991; Chabbert *et al,* 1993a, 1993b; Cone and Engels, 1993). When investigated, this result proved to be true for each part, or cell-wall part, of the plant such as the leaf blade, the sheath and the stem. Sheldrick (1979) demonstrated, using the Tr inbred, that the difference in lignin content under warm and sunny conditions could also be found when cropped in field conditions in the south of England, showing a reduction of 30 – 40% in *bm3* plants.

The genetic aspects of lignin content have also been investigated. During a comparative study of normal genotypes and double mutants, mainly involving the *bm1* gene, Miku *et al* (1980) noticed a significant influence of the normal genome background on the lignin content of the stalks and leaves, and genome x brown-midrib genes interactions. On 21 *bm3* late hybrids representing a 7-parent diallel and their isogenic normal counterpart hybrids, Gentinetta *et al* (1990) observed an average lignin content of *bm3* hybrids equal to 63.8% that of normal hybrids. The variability in lignin content between hybrids was similar for normal and *bm3* hybrids, showing a predominance of specific combining ability (SCA) effects over general combining ability (GCA), with a GCA/SCA ratio equal to 0.8.

Contents in hydroxycinnamic acids bound to lignin

Kuc and Nelson (1964) first established that, after the release of esterified aromatic compounds by mild alkaline hydrolysis, normal plants displayed, at all growth stages, a higher amount of *p*-coumaric acid than *bm1*, with only a small difference in the amount of ferulic acid. Similarly, alkaline hydrolysis of DMF lignin yielded equal quantities of ferulic acid from normal and *bm1* plants, but almost 50% less *p*-coumaric acid from *bm1* plants (Gee *et al,* 1968). Studying the DMF lignin of the stalks of the normal and *bm1* Tr inbred lines, Cymbaluk *et al* (1973) pointed out a higher content of *p*-coumaric and ferulic acids in the dry matter of normal Tr material than in the *bm1* tissues, with a most significant decrease of the *p*-coumaric acids in *bm1* plants. When comparing the 4 brown-midrib genes, Kuc *et al* (1968) noted that normal plants and brown-midrib mutants contained the same percentage of ferulic acid, whereas a higher content was found in double mutants. The yield of *p*-coumaric acid appeared to be reduced only for the *bm1* gene, and especially in the double mutants containing the gene *bm1*. Contrary to this study, the reduced yield of p-coumaric acid was later proved to be true for the *bm3* and *bm2* genes. Hartley and Jones (1978), studying the normal and *bm3* Tr inbred lines, observed in each of the vegetative organs (leaf blade, leaf sheath and stem) a ratio of *p*-coumaric/ferulic acid inferior in *bm3* plants compared to normal plants. Similarly, Cherney *et al* (1989), reported a lower *p*-coumaric acid content in *bm3* tissues compared to normal tissues of the fifth internode of the stem, whereas the ferulic acid content was similar for *bm3* and normal internodes. Finally, Chabbert *et al* (1993a) confirmed a lower ratio of *p*-coumaric/ferulic acid, after alkaline hydrolysis, in internodes of *bm3* plants as compared to normal plants; and from a comparison of non-isogenic materials, *bm2* plants appeared with an intermediate ratio of *p*-coumaric/ferulic acid between normal and *bm3* plants, closer to normal than to *bm3* plants (Chabbert *et al*, 1993b).

Lignin composition

In measuring the products of an alkaline nitrobenzene oxidation of lignin, Kuc and Nelson (1964) first pointed out that the yield of phenolic monomers was considerably reduced in *bm1* plants, about 4 times lower, indicating a larger amount of residue resistant to nitrobenzene oxidation, but the ratios of syringaldehyde, vanillin and *p*-hydroxybenzaldehyde to total aldehydes, and the ratio of syringaldehyde to vanillin, were the same for normal and *bm1* plants. Gee *et al* (1968) confirmed these results with DMF lignin, *bm1* plants yielding lower amounts of the 3 aldehydes after alkaline nitrobenzene oxidation. They also pointed out that the DMF lignin of the *bm1* plant contained more carboxyl groups than the DMF lignin of normal plants; *bm1* DMF lignin had more alkali-labile bonds. Studying the Tr inbred and its isogenic mutant *bm1* Cymbaluk *et al* (1973) also reported that the DMF-lignin of the *bm1* stalk tissues displayed less of the 3 aldehydes than normal plants, and particularly almost 2.5 times less syringaldehyde. When they compared the 4 brown-midrib genes, Kuc *et al* (1968) reported, after a nitrobenzene oxidation of the DMF lignin, a lower ratio of syringaldehyde to vanillin for the stalks of the *bm1* plants, but no difference between normal and *bm3* plants, and only slightly lower ratios for *bm2* and *bm4*. They also pointed out a higher release of *p*-hydroxybenzaldehyde from *bm3* and *bm4* stalks of the plants, as compared to normal or *bm1* and *bm2* plants.

As previously observed with *bm1* mutants, Grand *et al* (1985) found that, when the lignin was subjected to alkaline nitrobenzene oxidation, it also yielded a reduced amount of phenolic uncondensed monomers in *bm3* mutants in comparison with normal plants (20-d-old corn plants of normal and *bm3* inbred lines). They also demonstrated a reduction in the proportion of methoxylated units in the lignin, with a higher percentage in the release of *p*-hydroxybenzal-dehyde, which was lower for vanillin, and almost the same for syringaldehyde. They particularly emphasized the low catechol *O*-methyl transferase (OMT) activity of the *bm3* plants. This was confirmed by Cherney *et al* (1989) who reported a lower specific activity of OMT in meristems and tissues of *bm3* internodes, compared to normal internodes, harvested 58 d after planting. Lapierre *et al* (1988) performed the lignin characterization by analysis of the degradation products recovered from thioacidolysis, on internodes of normal and *bm3* maize plants, harvested at grain maturity. In this way, they revealed a drastic decrease of the syringyl/guaiacyl ratio in recovered products, in the case of *bm3* compared to the normal line (0.39 in *bm3* compared with 1.72 in normal plants), which emphasizes that it is essentially the biosynthesis of syringyl units which is affected by the *bm3* mutation. Similar results were reported by Gaudillière and Monties (1989). Lapierre *et al* (1988) also found evidence of an unusual compound, the 5-hydroxyguaiacyl unit, which is a characteristic of *bm3* plants and only found as traces in normal plants. This could suggest that the main step affected by the *bm3* mutation is the methylation of 5-hydroxyferulic acid, catalyzed by a catechol *O*-methyl transferase. Finally research undertaken by Chabbert *et al* (1993a) with 3 different normal and *bm3* maize hybrids showed a lower syringyl/guaiacyl ratio after thioacidolysis in *bm3* plants compared with normal plants, which was in agreement with the study of Lapierre *et al* (1988). A complementary result was recently obtained by Chabbert *et al* (1993b), with a higher syringyl/guaiacyl ratio in *bm2* plants, as compared to normal and *bm3* plants.

From previous studies, brown-midrib plants yielded lower amounts of monomeric units after alkaline nitrobenzene oxidation. The lignin of brown-midrib plants thus appeared as a more condensed polymer than the lignin of normal plants. Nuclear magnetic resonance spectra obtained on alkali-lignins and DMF-lignins extracted from corn stalks of the Tr inbred line, normal and *bm1* (Gordon and Griffith, 1973) revealed that the *bm1* lignin has a higher degree of cross-linking in the propane side chain of the lignin molecule than in normal lignin. Thus, the normal Tr line lignin seems to be a less-condensed polymer than the *bm1* one.

VARIABILITY OF CELL-WALL CONTENT AND CELL-WALL CARBOHYDRATE CONTENT OF THE NORMAL AND BROWN-MIDRIB MAIZE

Cell-wall content

Lechtenberg *et al* (1974) demonstrated, on segregating populations, that the cell-wall contents were nearly the same for *bm3* and for normal corn stover silages and stalks. However, El-Tekriti *et al* (1976) found that the cell-wall content appeared lower in the *bm3* stalks and leaves of segregating populations. This result was confirmed by Frenchick *et al* (1976) with a cell-wall content of 53.7% for the *bm3* silage (whole plants) and 59.5% for the normal. A2-yr study was conducted by Keith *et al* (1981), which showed that the cell-wall content did not differ between *bm3* and normal silage (whole plants) in yr 1 but that it was lower for the *bm3* in yr 2. Cone and Engels (1993) showed a significantly lower NDF content of a *bm3* hybrid, compared with its normal counterpart. The generally lower cell-wall content for the *bm3* plant resulted, at least in part, from a reduced lignin content.

Cellulose content

The cellulose content, as a percentage of dry matter, was estimated according to a Van Soest procedure in all the work reported here (Van Soest, 1963, 1965, 1967; Goering and Van Soest, 1970). According to Muller *et al* (1971), the cellulose content of the double mutant *bm1bm3* was inferior to that of *bm1*, *bm3* and normal plants. Using plants ensiled without ears, Muller *et al* (1972) found similar cellulose contents in normal and *bm3* mutants (28.9% and 29.0%). Lechtenberg *et al* (1972, 4 brown-midrib genes; 1974, *bm3*) demonstrated, with Tr inbred lines and on segregating populations, that the cellulose contents were nearly the same for brown-midrib and for normal maize stovers and stalks. In a 2-yr study, Keith *et al* (1981) found cellulose contents consistent with the first 2 results. Finally Weller *et al* (1984), working with normal and *bm3* maize plants of 3 genotypes (INRA 188, INRA 240 and INRA 258), which were harvested at 3 stages of maturity, and Cone and Engels (1993) with LG 11, which was harvested 9 times between mid-July and mid-November, found similar cellulose concentrations in both normal and *bm3* plants.

When the cellulose content was estimated through the crude fiber content, no significant difference was observed between normal and *bm3* genotypes (Frenchick *et al*, 1976; Inoue and Kasuga, 1989, 1990; Barrière *et al*, 1993).

Hemicellulose content

The hemicellulose content was estimated according to similar Van Soest procedures as for the cellulose content. The hemicellulose content was the same for normal, *bm1* and *bm3* plants, but with a trend for higher values in the *bm1bm3* plants (Muller *et al*, 1971). Using plants ensiled without ears, Muller *et al* (1972) found an hemicellulose content slightly higher for *bm3* than for normal plants (22.8 and 21.7%). When comparing the 4 brown-midrib genes, Lechtenberg *et al* (1972) reported that the stalks and stovers of brown-midrib mutants displayed a slightly higher percentage of hemicellulose. In the study of El-Tekriti *et al* (1976), the hemicellulose content was also higher in the *bm3* stalks and leaves than in normal ones. Cone and Engels (1993) later observed increasing differences in hemicellulose content, with a higher value for the *bm3* hybrid, when estimated as a percentage of the cell-wall content during the cropping season, and especially when related to the decreasing difference in cell-wall content between hybrids during ageing. However, for the first year of their study, Keith *et al* (1981) found no differences between *bm3* and normal silage for the hemicellulose content, whereas in the second year, the hemicellulose content was significantly higher for normal than for *bm3* silage; Weller *et al* (1984) reported similar hemicellulose concentrations in both normal and *bm3* plants.

COMPARISONS OF THE FEEDING VALUE BETWEEN NORMAL AND BROWN-MIDRIB MAIZE

In vitro *studies*

Barnes *et al* (1971) reported the first studies on a normal inbred and 3 brown-midrib genotypes (*bm1*, *bm3* and the double recessive mutant *bm1bm3*), which were developed in the Tr inbred line background, and harvested at 10, 35 and 55 d after silking, using a Tilley and Terry (1963) method. Research on the different plant parts has proved that the *in vitro* digestibility of the *bm3* vegetative organs (stem, leaf, sheath, cob, husk) was superior, whereas the *in vitro* digestibility of the grain was comparable. The *bm1* genotype was often closer to the normal type than to the *bm3*, and the *bm1bm3* type was intermediate, or similar to the *bm3*.

Lechtenberg *et al* (1972) were the first to compare the *in vitro* feeding values of the 4 *bm1*, *bm2*, *bm3* and *bm4* genes with the Tr inbred, which was harvested at 35 and 55 d after silking. Hybrid pools, segregating for the brown-midrib mutants *bm1*, *bm2*, and *bm3*, were simultaneously studied. Digestibility was particularly improved with the *bm3* and the *bm1bm3* Tr inbred, with a similar observation made for *bm3* hybrids. These results were in close correlation with the ADL content of the different genotypes. Whilst studying the Tr inbred and its isogenic mutant *bm1*, Cymbaluk *et al* (1973) found a higher *in situ* digestibility of the *bm1* stalks.

Lechtenberg *et al* (1974) demonstrated that the amounts of cell wall and cellulose digested during a 72-h *in vitro* study on segregating populations were considerably greater for *bm3* stalks than for normal ones. Similarly, El-Tekriti *et al* (1976) demonstrated that the *in vitro* dry matter disappearance, determined using the technique of Tilley and Terry (1963), was clearly higher in the organs (stems and leaves) of the *bm3* plants. Working with the Tr inbred, Hartley and Jones (1978), also demonstrated a greater enzymatic degradation of *bm3*. These results were confirmed by Weller *et al* (1984), who found that the *in vitro* digestible organic matter in the dry matter values were significantly higher for the *bm3* ($p < 0.05$) than for their normal counterparts, and by Gupta *et al* (1988) who reported that *bm3* lines had higher *in vitro* dry matter digestibility. Comparing *bm3* plants to normal ones, within the progeny of a 3-way hybrid including a normal line in the female single cross, Inoue and Kasuga (1989) pointed out a higher enzymatic digestibility of the organic matter of the stover, a little lower than 4 percent units. When comparing normal plants and *bm3* plants, Grenet and Barry (1991) demonstrated a faster rate of *in sacco* degradation of *bm3* genotypes. They outlined very different degradation processes with observations made by scanning electron microscopy. The histological structure of the 2 maizes was the same, but after 24 h in the rumen the parenchyma of the *bm3* maize degraded faster and the secondary walls of the fibers of its vascular bundles were degraded whereas those of normal maize remained intact. After 72 h in the rumen, the sclerenchyma of normal maize changed little, whereas the *bm3* maize had much thinner walls and was abundantly colonized by rumen bacteria. New results by Cone and Engels (1993), comparing a *bm3* hybrid and its normal counterpart harvested 9 times from mid-July to mid-November, showed that the difference in lignin content (ADL, as a percentage of dry matter) decreased during the latter part of the cropping season, after the harvest silage period, and similarly the *in vitro* rumen fluid digestibility (Engels and Brice, 1985) of the cell wall of the 2 types of hybrids were closer during that period. In connection with the apparently more condensed characteristic of the brown-midrib lignin polymer, Thorstensson *et al* (1992) considered that, with equal amounts of lignin, the *bm3* plant lignin could show an apparently greater inhibition to the digestion of polysaccharides than normal plant lignin. Studies performed by Köhler *et al* (1989, 1990) with normal maize hybrids and brown-midrib hybrids (the types of brown-midrib genes were not clearly indicated), seemed to show an increased digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose in some brown-midrib hybrids and, at the same time, a lower lignin content.

Experiments with sheep

Studying the Tr inbred and its isogenic *bm1* counterpart, Gordon and Neudoerffer (1973) found that *bm1* appeared to have a slightly higher digestibility of dry matter and fiber in sheep. The *in vivo* experiments undertaken on lambs by Muller *et al* (1972), using plants deprived of ears, showed increased intake by lambs of *bm3* corn silage and demonstrated that animals that were fed with *bm3 ad libitum* or rationed at 90% of *ad libitum* intake, showed an increase in cellulose digestibility of 8.7 and 6.9 percent units respectively, and similarly of 12.5 and 6.2 in hemicellulose digestibility (cellulose and hemicellulose according to Van Soest, as a percentage of dry matter). During a digestibility experiment (Stallings *et al*, 1982), sheep consumed significantly more *bm3* than normal silage dry matter (1.36 and 1.15 kg/d) and apparent dry matter digestibility was greater for *bm3* silage than normal (70.4 and 64.3%); the same was true for the apparent ADF digestibility (61.8 and 46.8%). During 2 tests, Weller and Phipps (1986) compared normal and *bm3* maize silages, which were fed to sheep. In both experiments, the *bm3* gene significantly increased the digestibility of the organic matter, cell-wall constituents, ADF and cellulose, whereas, in the first test, the digestibility of hemicellulose was greater with the *bm3*, but in the second test, the digestibility of the hemicellulose was similar in both silages. Barrière *et al* (1985), with results amounting to 10 years of experiments on sheep confirmed a greater intake of *bm3* (average 5.3%), an increase in organic matter digestibility (average 3.5%) and a great improvement in cellulose digestibility by an average of 15.6%. Recent studies undertaken by INRA at Lusignan have strengthened and refined these points (Barrière *et al*, 1993).

Experiments with goats

Whether fresh plants or silage were offered, the dry matter intake of goats was higher with *bm3* hybrids (114%) as compared to normal ones (100%); but the milk yield was similar with the 2 types of hybrids (Gallais *et al*, 1980). With whole-plant silage of normal maize plants and their *bm3* isogenic counterparts, Inoue and Kasuga (1990) reported that the digestibility of organic cell wall and crude fiber, when tested in digestion experiments on goats, was 16 and 11 percent units higher, respectively, for *bm3* hybrids; the digestibility of the residue of organic cell wall without crude fiber was also 15 percent units higher for *bm3* plants, indicating a significant improvement of the digestibility of both cellulose and hemicellulose in the cell walls of *bm3* maize.

Experiments with cattle

The first tests were conducted on heifers in 1972, 1973 and 1975 by Colenbrander *et al*. They noted superior intake and body weight gain in animals fed *bm3* silage, as opposed to normal genotypes. Keith *et al* (1981) conducted a study to compare the performances of feedlot cattle fed either *bm3* maize silage or its normal genetic counterpart with or without different levels of additional grain maize. They found that the feeding of *bm3* silage resulted in a significantly greater cattle body weight gain when no additional grain maize was fed, but no difference in body weight gain was observed when grain was fed at 2% of the body weight. Malterre *et al* (1985) demonstrated that *bm3* maize was better ingested in young bulls, allowing greater body weight gain when fed with similar supplements. When the supplements were reduced, the young bulls fed silage of *bm3* plants, greatly increased their intake and maintained a slightly superior body weight gain. Tests performed by INRA at Lusignan showed that zootechnic performances of young bulls fed with *bm1* maize silage were lower than those of young bulls fed with *bm1* maize, even if the *bm1* genotype was slightly more efficient than the normal genotype (Barrière *et al*, 1993).

A greater intake of *bm3* maize was observed in dairy cows in North America by Rook *et al* (1977), Sommerfeldt *et al* (1979), Block *et al* (1981), Stallings *et al* (1982), but similar observations were not conclusive in studies made by Frenchick *et al* (1976) or Keith *et al* (1979). Rook *et al* (1977) and Stallings *et al* (1982) found similar milk production between *bm3* and normal groups, while Frenchick *et al* (1976), Sommerfeldt *et al* (1979), and Block *et al* (1981) found slight trends to higher milk production in cattle fed *bm3* hybrids. A significant increase in milk was only observed once under US feeding conditions (Keith *et al*, 1979). Actual and fat-corrected milk yields were 1.3 and 0.9 kg greater for cows fed *bm3* silage than for cows fed normal silage at a forage-to-concentrate ratio of 72:25. Yields were 1.6 and 1.0 kg greater for actual and fat-corrected milk, for cows fed *bm3* hybrids compared with those fed normal silage at 60:40. Increased body weights of cows fed *bm3* corn silage were noted every time this trait was observed (Frenchick *et al*, 1976; Rook *et al*, 1977; Keith *et al*, 1979; Sommerfeldt *et al*, 1979; Stallings *et al*, 1982). It thus appeared that energy intake was apparently not limiting for milk production and it seemed that the extra nutrients available from the greater intake and digestibility were partitioned more into meat or fat body tissues than in milk production.

In the United Kingdom, the effect of feeding *bm3* silage to cattle was studied by Weller and Phipps (1986). In a 28-d trial, 6 Friesian cows, in weeks 27–30 of lactation, were offered a diet in which 30% of the dry matter consisted of concentrates and 70% was either normal or *bm3* silage. They confirmed a greater intake of the *bm3* maize and found that *in vivo* digestibility of the dry matter, organic matter, cell-wall constituents, ADF, cellulose and hemicellulose were higher in the *bm3* diet. They also reported that cows fed the *bm3* diet produced more milk (15.9 compared with 13.4 kg/d), with no difference in milk composition, and had a higher average daily live-weight gain.

Under French rearing conditions, with cows fed a *bm3* or normal silage with the same energizing supplements, Hoden *et al* (1985) confirmed better intake of *bm3* maize, but with only a small improvement in zootechnic performance. A reduction of 1.3 kg in the dried energizing supplements fed to animals receiving *bm3* silage allowed a large increase in the quantities being ingested and a higher production in milk.

MECHANICAL PROPERTIES AND AGRONOMICAL VALUES

While comparing *bm3* mutants and normal counterparts of 3 maize single crosses, Zuber *et al* (1977) pointed out that *bm3* plants had between 17 to 26% less crushing strength of the dry third internode; the weight of a given segment was lower but the thickness of the rind was similar. This was also confirmed by Weller *et al* (1985). Four normal hybrids and *bm3* showed higher resistance to crushing and greater stem weight in the normal genotypes but a similar rind thickness. Although the *bm3* gene slightly increased root lodging, susceptibility to lodging was mostly explained by the genetic background of the hybrids. Similarly the previously described study, by Gentinetta *et al* (1990), of 21 *bm3* hybrids representing the 7-parent diallel and their isogenic normal counterpart hybrids, highlighted that *bm3* hybrids had slightly more stalk breakage than normal plants, but the differences between normal hybrids were not significant, while a significant genetic variability existed between *bm3* hybrids. Some *bm3* hybrids had no more stalk lodging than their corresponding normal counterparts. Genotypes *bm3* appeared to be more susceptible to breakage, when equally infected with stalk-rot or infested with European corn-borer (Anglade, Gallais and Barrière, unpublished data). However, when breeding for a stronger resistance of *bm3* genotypes to lodging, Nesticky and Huska (1985) obtained lines that did not differ in puncture strength for stalk penetration as opposed to normal control.

Apart from the mechanical properties of the stems, the agronomical value of the brown-midrib genotypes seemed distinctively inferior to that of their normal isogenic counterparts and this was particularly noticeable for the *bm3* gene. This gene delayed silking by approximately 5 d, decreased the dry matter content at silage harvest and decreased the grain and biomass yields by up to 20% (Gallais *et al*, 1979, 1982; Weller *et al*, 1985). Inoue and Kasuga (1989) also pointed out a significant decrease of the grain and biomass yields of *bm3* plants, but they observed no difference for early vigor and no variation of earliness, neither at tasseling or silking time, nor for days to yellow ripe stage. Miller and Geadelmann (1983) have observed lower seedling dry weights and lower dry matter accumulation rates during vegetative growth in S1 *bm3* lines compared with the normal S1 lines, using pools with narrow base germplasm. Furthermore, after topcrossing, S1 *bm3* progenies produced lower grain and whole plant yields, 77 and 90% respectively, when compared with normal S1 progenies (Miller *et al*, 1983). Recurrent selection attempted on original *bm3* silage genotypes was unable to yield material with reasonable agronomical value (Barrière *et al*, 1988b), but there might be a possibility for improvement from pedigree breeding from entries of a very high agronomic value (Barrière *et al*, 1988a, 1993).

Studies on 15 *bm3* late hybrids, representing a 6-parent diallel and on their 15 isogenic normal counterpart hybrids, were undertaken in Hawaii (Lee and Brewbaker, 1984). They observed a grain yield and a stover yield in *bm3* hybrids of 80 and 83%, respectively, of the normal hybrids, without modification of the silking dates. The leaf area index (LAI) of *bm3* hybrids was not significantly inferior to that of normal plants, whereas the stem diameter of *bm3* plants was significantly inferior. For grain yield, the GCA effects were similar between normal and *bm3* plants, but for stover yield the GCA effects were lower in the *bm3* hybrids. It was also observed that the SCA effects were inferior for *bm3* hybrids. A significant predominance in GCA effects appeared in all cases. All hybrids that have been tested showed a decrease in whole plant yield of at least 10% over the normal types. Similarly Gentinetta *et al* (1990) observed on the previously described diallel that the whole plant yield of the *bm3* hybrids was equal to 84% of the normal ones, but with a slightly superior earliness in *bm3* hybrids. Contrary to what had been previously observed and following the observations made by Lee and Brewbaker (1984), the decrease in yield affected on average the grain part as much as the stover parts. The variability in productivity was significantly superior among normal hybrids, with mean squares nearly double for whole plant yield, and a particularly low mean square for the stover yield of *bm3* hybrids. This mean square was lower than expected from a proportional decrease of yield of each hybrid. In the same way the GCA/SCA ratios were superior in normal hybrids, due to the higher GCA values and the proportionally lower SCA values. Out of 21 *bm3* hybrids, 3 displayed a whole plant yield equal to 95% of the normal corresponding plants; one of them even appeared slightly superior. Moreover, these 3 hybrids corresponded to the sub-diallel of 3 inbred lines (B77bm3, C103Dbm3 and H28bm3).

DISCUSSION AND CONCLUSIONS

The effects and characteristics of the 4 brown-midrib genes were not equally investigated. Preliminary work dealt either with the *bm1* gene, or with all 4 genes. From 1973 to 1974, most work only dealt with the *bm3* gene, and this is probably due to the results of Barnes *et al* (1971) and Lechtenberg *et al* (1972) proving a higher digestibility of *bm3* plants, as compared to normal ones or to other brown-midrib ones.

Some typical characteristic of brown-midrib genotypes can be pointed out, as illustrated in table I from papers giving comparisons of the different brown-midrib genes. It is worth noticing that the results of Kuc *et al* (1968) may appear contradictory to those obtained later, especially for some characteristics of lignin of *bm3* maize.

The brown-midrib mutants are mainly recognized by their reduced lignin content, even though the ranking of the genes for this feature and the importance of the decrease may depend on the way the lignin is measured and of the genetic backgrounds of the plants. This reduction in lignin content appears even more impressive in some double mutants, although this still needs to be confirmed. Differences in the lignin composition and in the ratios of the esterified phenolic acids are also conclusively demonstrated. The amount of esterified ferulic acid appears to be nearly similar in both normal and brown-midrib plants, while the *p*-coumaric acid amount is lower in *bm1* and *bm3* plants. According to the different papers, the release of aldehydes after use of nitrobenzene oxidations is much lower in *bm1* and/or *bm3* lignins, as compared to normal lignins. Brown-midrib lignin appears as a more condensed polymer than the normal one. At the same time, the ratios of syringaldehyde to vanillin are different in normal and brown-midrib plants. After thioacidolysis, the lignin also yields lower syringyl/guaiacyl ratios in *bm3* plants compared with normal ones. Moreover, *bm3* plants are also characterized by an original 5-hydroxy-guaiacyl unit, which appears after thioacidolysis as well as through a lack of catechol *O*-methyl transferase activity.

Finally, after *in vitro* experiments pointing out the higher digestibility of *bm3* plants, the interest of the brown-midrib genotypes was established because they provide greater intake and digesti-

Table I. Some characteristic results illustrating the effects of the 4 brown-midrib genes of maize.

Reference	*Character studied*	*normal*	bm1	bm2	bm3	bm4
Kuc *et al*, 1968	DMF lignin, stalks [a]	24.5	21.0	22.0	21.0	21.4
Lechtenberg *et al*, 1972	ADL, stover, % dry matter [b]	7.8	7.1	6.8	5.3	7.3
Chabbert *et al*, 1993b	Klason lignin, stalks [c,d]	16.0	(12.6)[k]	10.9	12.0	–
Kuc *et al*, 1968	*p*-coumaric/ferulic [e]	3.6	1.7	2.9	3.1	3.3
Chabbert *et al*, 1993b	*p*-coumaric/ferulic [cf]	4.6	(4.8)[k]	3.6	2.2	–
Kuc *et al*, 1968	syringaldehyde/vanillin [g]	2.4	1.6	2.0	2.4	1.9
Chabbert *et al*, 1993b	syringaldehyde/vanillin [gh]	1.0	–	1.4	0.3	–
Chabbert et al, 1993b	syringyl/guaiacyl [c,i]	1.1	(1.1)[k]	3.1	0.3	–
Lechtenberg *et al*, 1972	IVDMD [j]	46.4	47.0	51.8	54.8	51.3

[a] DMF lignin as a percentage of the cell wall; [b] ADL of stover from Tr inbred lines harvested 55 d after silking; [c] average data of top, middle and bottom internodes of the stalks: [d] Klason lignin content expressed as percentage of cell-wall residue; [e] phenolic acids released by alkaline hydrolysis estimated as mg/g in lignin; [f] phenolic acids released by alkaline hydrolysis expressed as μmol/g of the cell-wall residue; [g] products of alkaline nitrobenzene oxidation estimated as mg/g in lignin; [h] products of alkaline nitrobenzene oxidation expressed in μmol/g of Klason lignin; [i] products of thioacidolysis expressed as μmol/g of Klason lignin ; [j] IVDMD (*in vitro* disappearance of dry matter) according to Tilley and Terry (1963); [k] data for *bm1* materials (Chabbert and Tollier, personal communication) were obtained from separate comparisons of normal, *bm1* and *bm3* plants. All the values of normal and *bm3* plants were similar in the 2 experiments.

bility, and a higher rate of digestion when they are fed to cattle. It is worth noticing that the improvement of digestibility or efficiency observed with cattle fed *bm3* silage was higher than expected from *in vitro* measurements of digestibility. In this way, Inoue and Kasuga (1989-1990) showed a higher *in vitro* enzymatic digestibility of the stover of *bm3* plants reaching only 4 percent units compared with normal ones, while the increase of organic cell-wall digestibility estimated from experiments on goats reached 15 percent units. Similarly, from experiments with dairy cows performed in Lusignan (Hoden *et al* 1985), the higher energy content of *bm3* hybrids did not clearly appear from *in vitro* tests, but only from feeding cattle with diets involving a reduced content of energizing concentrates.

However, the *bm3* gene delays maturity, increases lodging and/or breakage susceptibility, because of poorer mechanical properties of the stem. It also decreases grain and stover yield. But these observations also appear to be genotype-specific. This genetic variability suggests the possibility of producing brown-midbrid genotypes as good as the normal ones, provided well-adapted breeding methods are used, with lines of a very high agronomical value, such as those used in the best and newly registered hybrids.

From a theoretical point of view, working with brown-midrib plants, and comparing the effects of each of the genes, is probably a major step towards understanding the lignification in gramineous plants, and the relationship between lignification and digestibility. Molecular biology, cloning of *O*-methyl transferase and cinnamylic alcohol dehydrogenase enzymes, use of the anti-sens RNA techniques, and cloning and sequencing brown-midrib loci of normal and mutant genotypes could be also powerful tools towards understanding lignification. Some criteria, that could be useful for plant breeders of normal silage maize, may result from theoretical work with brown-midrib maize, perhaps even before brown-mibrid hybrids with good agronomic characteristics are available.

ACKNOWLEDGMENTS

This paper is a contribution to the INRA network Agro-bio–lignine. We acknowledge B Monties, B Chabbert, MT Tollier and C Lapierre for their help in our training in the biochemical characteristics of maize cell walls. We thank M Rives for his helpful criticism. We also acknowledge A de Pourtalès for the linguistic revision of this manuscript.

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